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The Use of Liposomes from Soya Phospholipids in Cosmetics

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Introduction

Phospholipids are the most important components of biological membranes, i.e. they exist ubiquitously in nature. The most important phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine in addition to a number of other accompanying phospholipids existing in smaller quantities, whose contents may be very different depending on organism.

Animal and vegetable phospholipids differ in their fatty acid content. Egg phosphatidylcholine, for instance, contains 28–38% palmitic acid, 9–18% stearic acid, 25–37% oleic acid, 12–17% linoleic acid, about 0.5% linolenic acid and 1–7% arachidonic acid. Soya phosphatidylcholine, depending on provenience, contains 10–15% palmitic acid, 1–5% stearic acid, 6–13% oleic acid, 61–71% linoleic acid and 4–7% linolenic acid. Hydrated phospholipids which are produced from the natural phospholipids by catalytic hydrogenation contain, accordingly, a very high percentage of stearic acid in addition to a smaller percentage of palmitic acid.

Vegetable phospholipids are predestinated particularly for topical applications in cosmetics and dermatology, since they have the highest content of esterified essential, i.e. polyunsaturated fatty acids (»vitamin F«), especially linoleic acid. Linoleic acid is one of the most valuable cosmetic active ingredients (1), the shortage of which causes increased comedogenity in the skin (2). The transdermal moisture loss is inversely dependent on the linoleic acid content of the skin: the supply of linoleic acid increases, within a short time, the barrier function of the skin and decreases the water loss (3, 4, 5, 6). Soya phospholipids or other vegetable phospholipids, due to their surface activity and their ability to form liposomes, are thus the ideal form of transport for linoleic acid. Consequently, phospholipids in the skin (for the natural content of the stratum corneum see (7)) are highly responsible for the moisture household, promote the skin breathing, protect the skin from degreasing by detergents (8) and thus influence the softness and elasticity of the skin (2).

Why liposomes from soya phospholipids?

According to their provenience from biological membranes, the phospholipids produced by various extraction and cleaning steps have a »natural« tendency to renewed membrane (bilayer) formation in the aqueous environment. Therefore, liposomes as copies of the cell membranes are one of the most natural formulations of the phospholipids. This formulation largely meets the requirements of the cosmetics developers, since it leads to particularly good penetration and distribution of the liposomes on the one hand and of the linoleic acid contained in the soya phospholipids as an active ingredient on the other hand (see below).

Like the biological cells, the liposomes contain an aqueous interior volume which can take up active ingredients soluble in water (salts, moisturizing factors, etc.). The bilayer itself can store amphiphilic and lipophilic active ingredients (proteins, vitamins, oils, etc.). The inherent effect of liposomes from hydrogenated phospholipids, which is naturally smaller due to the absence of essential fatty acids, can thus be improved by »loading« with active ingredients. The high inherent effect available in liposomes produced from soya phospholipids can of course be increased even further by »loading«.

Basically it can be stated that, due to higher fluidity and the resulting higher permeability, the active ingredients are set free more quickly from loaded liposomes produced from soya phospholipids than from those produced from hydrogenated phospholipids (9). This again has its parallelism in the living cell: if the percentage of saturated phospholipids or/and cholesterin rises in the membrane, one will find a reduction of permeability (10). On the other

hand, the fluidity and permeability of the membrane will be increased if phospholipids with a high content of combined essential fatty acids are incorporated in the membrane. The consequences are still largely unclarified today. It is, however, an established fact that certain transport processes and functions of membranes come to an end when the fluidity of the membrane drops below certain limits (11).

An argument frequently used to advise against the application of natural phospholipids in cosmetics is their sensitivity to oxidizing influences (12, 13). Experience gained with the native oils, which are used more and more at present and have identical characteristic in this respect, shows however that the active ingredient idea gains more and more ground (2) and that the quicker decay (after opening the container), which is typical of a natural product, is accepted as »natural« by the consumer. On the other hand, the formulations can be stabilized by »vitaminisation« with vitamin E, vitamin C and vitamin A, singly or in combination, these vitamins constituting valuable cosmetic active ingredients and acting synergistically with phospholipids (1). Filling in dispensers or tubes is recommended as an alternative or in addition.

Criteria for selection of liposomes to be used in cosmetics

The cosmetics developer who has decided to use liposomes in his preparations is faced with a number of questions regarding the raw product. Even the question of using a finished liposome formulation or liposomes of one's own production by methods commonly used at present (film method, ultrasonic method, high-pressure homogenisation, microfluidizer, French-Press, reversed phase evaporation, dialysis of mixed micelles), which are described in detail in the literature (9, 14, 15, 16), is very difficult to answer in the normal case. Therefore, in addition to setting a precise target, it is important to have a number of questions answered by liposome suppliers or equipment manufacturers:

1. Are empty liposomes or loaded liposomes to be used?
2. If empty liposomes are used: Is it intended to achieve a maximum of cosmetic inherent effect (phospholipids of vegetable origin) or play the marketing aspects and oxidation stability a greater role (hydrogenated phospholipids)?
3. Is it important to create an image of naturalness, i.e. is a natural or even »edible« cosmetic product aimed at?
4. Are the liposomes compatible with all ingredients of the final formulation?
5. Where loaded liposomes are used: Is importance attached to the capacity of storing hydrophilic, amphiphilic and lipophilic substances? If so, is it necessary to clarify this question in connection with physical stability and homogeneity of the liposomes. According to previous experience it seems that an optimum is achieved with oligolamellar (up to 10 shells) liposomes.
6. Is it possible to guarantee an average size of the liposomes, is this size within the range of 100–400 nm, which is of interest for cosmetic purposes, or within the ideal fine range of 100–200 nm?
7. Are results available on stress tests with the liposomes (swing tests 5°/40°C; freeze-thaw cycling)?
8. Does the liposome preparation contain stabilizers (e.g. glycerol, cholesterol, dicetyl phosphate)? If so, are they physiological ones? Are carry-over effects to be expected, by preservatives, for instance?
9. Does the liposome preparation contain production-process-induced residual solvents, residual detergents or heavy-metal contaminations?
10. Are dermatological tests of the liposome base material and the liposome formulation available?
11. If loaded liposomes are to be used: Is the »loading« cosmetically and dermatologically safe, i.e. can risks due to the special kinetics of the liposomes be safely excluded? This point is also important for the future selection of preservatives and perfumes, since these are mostly of an amphiphilic or lipophilic composition, accordingly they may partly diffuse into the liposomes, and thus penetrate similarly well during the topical application.
12. Is a receipt inspection of the liposome preparation and a liposome-specific final inspection of the finished product (electron micrograph etc.) ensured internally or externally?
13. To what extent can liposomes be made available by the supplier and/or what is the capacity of any given equipment?
14. What is the production cost and/or purchase price of liposome dispersions?

Possibilities for application of liposomes from soya phospholipids in cosmetics

At present, liposomes are mostly contained in skin gels or skin creams. Particularly in the latter products there may be stability problems due to diffusion of tensides from emulsion into the liposome, for instance, and amphiphilic substances in general as well as due to interactions with lipophilic substances. A well-known phenomenon, for instance, is the conversion of large multi-lamellar (multi-shell) liposomes via large unilamellar (single-shell) ones into small unilamellar liposomes and ultimately their change into mixed micelles as a function of the tenside concentration (17). Generally, the best way of applying liposomes as natural moisture regulators (18) is adding them to any kind of gel-like preparations. What is of advantage is their prolonged action (19). At the same time, however, one should also consider uses in preparations in which alcohols are now used as solvents and coolants and can be replaced by liposomal formulations (e.g. after-shaves, fitness frictions, antiseptic solutions). Uses of interest may also be face- and hair-lotions, mouth-washes as well as formulations against pregnancy streaks, after-sun gels and the encapsulation of perfumes. Even such applications which seem to be exotic at the present time, such as hair rinsers (here, too, soya phospholipids in the form of liposomes have considerable cosmetic inherent effects: refattening of the scalp, hair conditioning and hair softening effect (20)) and oil baths (cf. (2)) with dermatologic pretension (optimal supply of linoleic acid to large skin areas, irritation-relieving effect; no additional synthetic emulsifier required, even in the case of »loading« with essential oils; no fatty residues on the bath tub surface) should be considered as the technical simplification of the liposome production continues. The inclusion of enzymes for adhesion of the liposomes to the keratinocytes, the encapsulation of additional moisture-maintaining substances, the inclusion of deodorizing substances, light protection agents and the use in antiperspirants have already been described (19, 21). Also of interest is the storage of native oils, which, like the soya phospholipids, contain essential fatty acids in esterified form (triglycerides) as well as natural vitamins and other caring substances.

Characterization of liposomes

The characterization, i.e. the determination of the size, the size distribution, the lamellarity, the inclusion and leakage rate as well as the detection of the liposomes in a final for-

mulation is very complicated. Experience shows that, in addition to well-known methods such as the Laser light scattering, the nuclear resonance spectroscopy, the gel chromatography, etc., which are described in detail in the literature (13, 9), the electron microscopy, as a selected method, is the most important and most accurate source of information for the developer.

The freeze-fracture technique now constitutes a routine preparation in electron microscopy and is very suitable for examination of liposomal formulations. In this process a drop of a liposome dispersion is placed between two small copper plates (sandwich), then it is fixed in liquid propane (Cryo Jet, Balzers) and subsequently broken in a freeze-fracture apparatus (BAF 400, Balzers). After shadowing with platinum and carbon, the replica can be examined in the transmission electron microscope. The advantage of this method is that the liposomes can be detected and examined in their respective environment (aqueous dispersion, gel, emulsion, etc.). *Fig. 1* shows the result of such a freeze-fracture preparation of a 10% liposome dispersion in an aqueous, physiological environment. The liposome base material consists of concentrated soya phosphatidylcholine. The liposomes do not contain stabilizers, except the natural vitamin E subsequently standardized to 0.02%. The liposomes are of oligolamellar structure. These liposomes are very stable: stress tests (5°C/40°C, 4 weeks, 24-hour change; 3 freeze-thaw cycles, −15°C/+20°C) with no objection.

Recent developments in the cryotechniques in electron microscopy permit using the bare-grid method (22) for characterizing liposome dispersions. In this process a drop of a liposome sample is placed on a carrier net and then sucked off as far as possible. The sample is then cryofixed by shooting into liquid ethane (Zeiss cryo-box with cryo-transfer) and transferred into a transmission-cryo-electron microscope. To ensure high-contrast representation of the frozen, non-contrasted sample, an electron energy spectrometer is used (Zeiss EM 902).

Fig. 2 is the photograph of a commercial 10% empty liposome concentrate from concentrated soya phosphatidylcholine in a physiological carrier medium. The concentration is identical with that described in *Fig. 1* (23). Liposomes cryofixed according to the bare-grid method and represented according to the electron-microscopical method in analogy with *Fig. 2* can be evaluated by means of a suitable picture analysis within a very short time. Unlike all other methods mentioned above, this method gives an exact distribution according to size, lamellarity, form and possibly even loading of the liposomes. An additional advantage of this method is the detection of different vesicles (e.g. oil droplets) and the fact that pictorial documentation is made available.

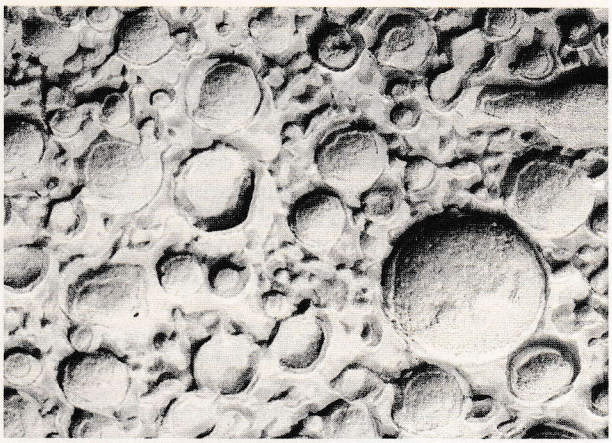

Fig. 1 Freeze-fracture preparation of a liposome concentrate (10%)
Magnification: ⊢——⊣ 0,1 µm

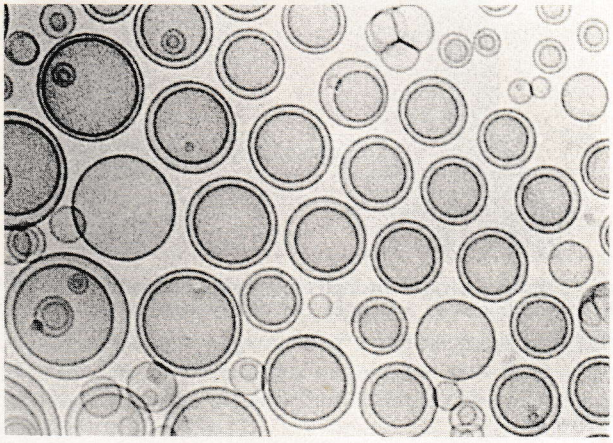

Fig. 2 Cryofixed liposomes (10% concentrate, diluted 1:10 with water)
Magnification: ⊢——⊣ 0,1 µm

Penetration of liposomes in topical applications

Liposomes, when compared with classical emulsions, penetrate very uniformly the entire horny layer (12). Dermatological examinations show that the concentrations of pharmaceutical active substances, such as hydrocortisone, when a liposomal formulation is applied, are 4–8 times higher in the epidermis and 9–14 times higher in the dermis in comparison with a w/o emulsion (24). For topical pharmaceutical formulations this means that a better transport takes place, active substance can be saved and, possibly, side effects can be reduced as well. Similar results have been achieved in tests with the application of triamcinolone (2 − ^{14}C) acetonide (25). Applied to cosmetics, this means that »loading« of liposomes must be handled with extreme caution. The »loadings« must be definitely safe, which of course applies to the composition of the empty liposomes as well. Therefore, suitable substances are only those, whose kinetics or harmlessness in the case of possible resorption by the skin are known, and with which years of experience have been gained with the use in »classical« formulations, and for which at least partial resorption has been assumed up to now. Where »new types« of loadings are intended, detailed toxicological investigations into the compatibility of the final formulations will be inevitable.

This estimation is confirmed by a study conducted by the Institute for Medical Balneology and Climatology of the University of Munich (26). This study deals with a liposome concentrate of identical basic composition as that specified in the description of *Fig. 1* and *2* – but loaded with monoclonal antibodies – applied to the skin of a pig. Thirty minutes later the antibody complex could be detected by specific colouration (APAAP technique) in the epidermis as well as in the dermis and its deeper regions. The study conducting the test on pig-skin is of a qualitative character and will have to be quantified by further investigations. For possible interactions between liposomes and cells see (14).

Abstract

This article shows that on the one hand the development of the liposomes technology offers great chances for many

new cosmetic products (which are very desirable from the consumer's point of view), but that on the other hand the cosmetics developer has to deal very intensively with questions of raw material selection, characterization of raw and finished products as well as the question about the biological fate of his formulations, in order to avoid risks. In this connection, soya phospholipids in the form of liposomes have much to recommend them, in view of their wide spectrum of activity and the decades of experience with these natural raw materials in food and pharmaceuticals.

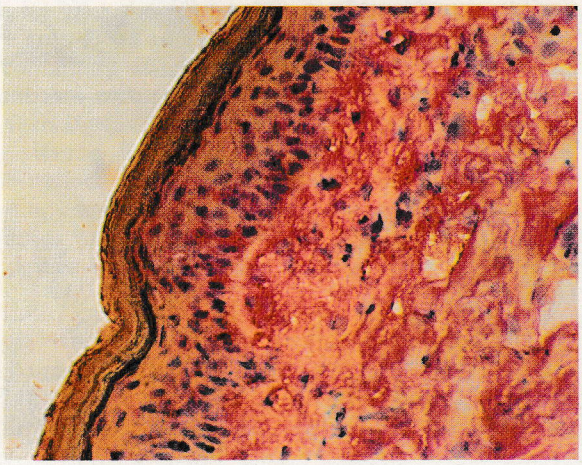

Fig. 3 shows a section through the pig-skin, magnified 400 times. The red colouration indicates the distribution of the antibody complex

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